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FURTHER EVIDENCE OF A POSSIBLE CENTRAL NERVOUS SYSTEM ROLE FOR --ETC(U)  
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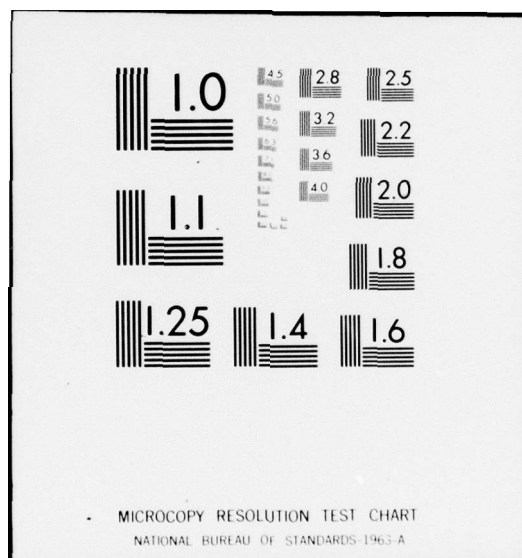
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) LEM obtained from glycogen-induced peritoneal exudates in rabbits was injected intracerebroventricularly to determine if the mode of action of LEM was via the monoamines (norepinephrine and serotonin), acetylcholine or prostaglandins. LEM given ivc produced fever, lowered plasma zinc levels and increased plasma $\alpha_2$ -MFP concentrations. LEM, $\Delta$ LEM and saline injected icv were all observed to have a nonspecific effect on blood neutrophils and lymphocyte concentrations. Pretreatment with atropine, $\alpha$ -MPT and p-cholorophenylalanine all failed to		

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modify the zinc depression and fever effects of LEM. However, they did significantly inhibit the synthesis of plasma  $\alpha_2$ -MFP. Pretreatment with prostaglandin synthesis inhibitors, indomethacin, acetylsalicylic acid and acetaminophen, increased the latency of the fever without effecting zinc or  $\alpha_2$ -MFP. Administration of these prostaglandin synthesis inhibitors during the fever slightly lowered the fever response for approximately 30 min but failed to alter zinc or  $\alpha_2$ -MFP. These data suggest that a balance between the neurotransmitters is required for certain of the metabolic changes after icv LEM without affecting the fever response. However, antipyretics appear to influence only the fever response associated with icv LEM but not the metabolic changes.

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~~Further~~ Evidence of a Possible Central Nervous  
~~System~~ Role for Leukocytic Endogenous Mediators (LEM)

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Polymorphonuclear leukocytes (PMN) can be stimulated to release materials (leukocytic endogenous mediators; LEM) that on injection into rats will elevate serum acute-phase globulins (1,2), lower plasma iron (3) and zinc (4), elevate copper (1) levels, release neutrophils from the bone marrow (5), and cause a flux of amino acids from serum to the liver (2,6).

Previous studies have also shown that a variety of alterations in host metabolism can be induced by the administration of minute doses of LEM into the central nervous system (7,8). When rabbit LEM (10-50  $\mu$ l; ineffective systemic doses) is administered to rats by the intracerebroventricular (icv) route, lowered plasma iron and zinc, increased serum copper and  $\alpha_2$ -macroglobulin ( $\alpha_2$ -MGP), caused a flux of a nonmetabolizable amino acid to the liver, and increased body temperature (8). Current speculation suggests that pyrogen- and LEM-induced fever is mediated by the action of prostaglandins within the central nervous system. This speculation is based on the finding that inhibition of prostaglandin synthetase is the mechanism underlying the action of the antipyretics. However, there is evidence that monoamines (norepinephrine, epinephrine, and serotonin) are also involved in the control of body temperature (9).

The present study was undertaken to determine if inhibitors of prostaglandin synthesis or monoamines were primarily responsible for mediating the ability of LEM to regulate body temperature and certain other metabolic parameters in rats.

Materials and methods. Healthy, male Fisher-Dunning rats, weighing 200-250 g were used in this study. The rats were stereotactically implanted with a guide cannula (Plastic Products Co., Roanoke, Va.) aseptically into the right lateral ventricle and injected 12 days after the operation with 20  $\mu$ l of LEM; rectal temperature was recorded according to the procedure of Bailey *et al.* (8). All rats were bled via the orbital sinus 0, 5, and 24 hr after LEM inoculation, collecting approximately 1 ml of blood in 50 units of sodium heparin. A differential and white blood cell count was determined from the heparinized blood sample. The samples were then centrifuged for 20 min at 2500 g and the resulting plasma collected. Plasma zinc concentration was determined by atomic absorption spectrophotometry (10). Alpha<sub>2</sub>-macroglobulin was determined by the method of Eddington *et al.* (11). Statistical analyses were done using Student's *t* test for paired or unpaired variates.

LEM was prepared from rabbit PMN ( $1 \times 10^8$  PMN cell/ml) obtained from glycogen-induced peritoneal exudates as previously described (2). In the present experiment 20  $\mu$ l of LEM was ineffective when administered ip or iv. Heat-inactivated LEM ( $\Delta$ LEM) was the supernatant obtained from placing active LEM in a boiling water bath for 30 min and then centrifuging at 2500 g for 5 min.  $\Delta$ LEM did not cause fever. The following drugs were administered, aspirin (acetylsalicylic acid; 25 mg/kg) dissolved according to the method of Feldberg and Saxena (12), indomethacin (2.0 mg/kg) dissolved according to the method of Clark and Cumby (13), and acetaminophen (50 mg/kg; APAP) dissolved in saline. All antipyretics were administered intraperitoneally (ip) 30 min prior to icv LEM or during the hyperthermic response. P-chlorophenylalanine methyl ester (100 mg/kg, administered ip at 48, 24 and 16 hr prior to LEM) was



dissolved in saline. Alpha-methyl-p-tyrosine methyl ester was dissolved in saline and a dose of 200 mg/kg was injected ip 16 hr prior icv LEM. Two ip injections of atropine (4 mg/kg) dissolved in saline was given 30 min prior and 3 hr after icv LEM administration.

Results. There appears to be a nonspecific shift in peripheral blood neutrophils (increase) and lymphocytes (decrease) after an icv injection of either LEM,  $\Delta$ LEM or saline (data not shown).

The data in Fig. 1 show changes in body temperature following icv injection of LEM (20  $\mu$ l). LEM caused a rapid fever within 60-90 min as previously described (8). The effect of pretreatment with  $\alpha$ -methyl-p-tyrosine ( $\alpha$ -MPT; inhibits the synthesis of catecholamines), p-chlorophenylalanine (PCPA; inhibits the synthesis of serotonin), and atropine (anticholinergic agent) on the fever activity of LEM are also shown in Figure 1. All these drugs failed to significantly inhibit fever production by LEM. However, LEM fever was enhanced in rats pretreated with PCPA.

The effect of  $\alpha$ -MPT, PCPA, and atropine on plasma zinc and  $\alpha_2$ -MFP after the administration of LEM are presented in Table I. LEM caused a significant decrease in plasma zinc 5 and 24 hr after treatment, when compared to 0 time. Atropine, PCPA, and  $\alpha$ -MPT, did not significantly inhibit the LEM depression of plasma zinc. Twenty-four hours after the icv injection of LEM the plasma concentrations of  $\alpha_2$ -MFP were increased significantly compared to 0 time. However, treatment with these drugs caused a significant decrease in the  $\alpha_2$ -MFP response when compared to LEM alone.

The effect of antipyretics administered during the maximum fever response of LEM are illustrated in Fig. 2. The fever produced by icv

injections of LEM was significantly depressed by APAP and indomethacin for approximately 30 min before the fever began to return (Fig. 2). Aspirin showed an insignificant reduction in the fever response. Orbital bleeding of these rats at 0, 5, and 24 hr revealed no difference in zinc or  $\alpha_2$ -MFP concentrations from that of LEM (Table II). APAP, aspirin, and indomethacin caused no significant changes in plasma zinc and  $\alpha_2$ -MFP. Administration of the antipyretics 30 min prior to an icv injection of LEM did not abolish the fever response (Fig. 3), but pretreatment did increase the latent period of the fever responses when compared to LEM. There were no changes in the LEM-induced responses in plasma zinc and  $\alpha_2$ -MFP concentrations in rats pretreated with the antipyretics (data not shown).

Discussion. In the present study LEM ( $1 \times 10^8$  PMN/ml; 20  $\mu$ l) was found to cause fever when injected into the lateral cerebral ventricle of the rat as previously reported (8). It has been proposed by some investigators that the neurotransmitters, norepinephrine, dopamine, and serotonin, and acetylcholine present in the brain are concerned with thermoregulation. Hellon (9) reported that chemically induced alterations in the concentrations of catecholamines can profoundly modify pyrogen-induced fever. The present study demonstrates the lack of involvement of the monoamines after the administration of LEM. The depletion of serotonin (with PCPA) or norepinephrine (with  $\alpha$ -MPT) concentrations in the brain had no apparent inhibitor effect on the development of fever after icv administration of LEM. These data are in agreement with depletion studies in other animal models (14,15). However, Harvey and Milton (16) reported that PCPA reduced the fever produced by icv pyrogen in cats. In contrast, PCPA pretreatment in

rats enhanced the fever response produced by LEM. A similar observation with PCPA has been demonstrated in rabbits after the administration of a leukocytic pyrogen (17). Teddy (18) provided evidence that  $\alpha$ -MPT significantly reduced the height and duration of fever in rabbits after endogenous pyrogen administration. In the present study, atropine was observed not to alter the fever response. Conversely, icv injected atropine, (200  $\mu$ g) in rabbits reversed the fever after a leucocyte pyrogen (19). It appears that species difference and the degree of depletion may influence the interpretation of these experiments.

Recent evidence has implicated prostaglandins in the fever response to leukocytic factors (9). The fever response to LEM in the present study appeared to be mediated via an increase in prostaglandin synthesis, since indomethacin, acetaminophen, and aspirin reduced the fever. Pretreatment with antipyretic agents delayed the fever further, suggesting prostaglandin involvement, thereby confirming similar observations in rats made by the other investigators with various prostaglandin synthesis inhibitors.

Alterations in plasma zinc and  $\alpha_2$ -MFP concentrations after icv treatment with LEM have recently been documented (8). The present study confirms the earlier observation that icv-injected LEM caused a significant decrease in plasma zinc and elevated  $\alpha_2$ -MFP levels. Pretreatment or injecting the antipyretics during fever did not block icv LEM affects on plasma zinc or  $\alpha_2$ -MFP. The antipyretics appear to influence only the fever responses, not the metabolic parameters. The observed suppression of a typical increase in plasma concentrations of  $\alpha_2$ -MFP caused by  $\alpha$ -MPT, PCPA and atropine when given after icv LEM suggest that a balance between the neurotransmitters is needed

for the  $\alpha_2$ -MFP response, but this balance is apparently not necessary for the zinc response. The data further indicates a possible role of the central nervous system during infection.

Summary. LEM obtained from glycogen-induced peritoneal exudates in rabbits was injected intracerebroventricularly to determine if the mode of action of LEM was via the monoamines (norepinephrine and serotonin), acetylcholine or prostaglandins. LEM given icv in minute doses produced fever, lowered plasma zinc levels and increased plasma  $\alpha_2$ -MFP concentrations. LEM,  $\Delta$ LEM and saline injected icv were all observed to have a nonspecific effect on blood neutrophils and lymphocyte concentrations. Pretreatment with atropine,  $\alpha$ -MPT and p-cholorophenylalanine all failed to modify the zinc depression and fever effects of LEM. However, they did significantly inhibit the synthesis of plasma  $\alpha_2$ -MFP. Pretreatment with prostaglandin synthesis inhibitors, indomethacin, acetylsalicylic acid and acetaminophen, increased the latency of the fever without effecting zinc or  $\alpha_2$ -MFP responses. Administration of these prostaglandin synthesis inhibitors during the fever slightly lowered the fever response for approximately 30 min but failed to alter zinc or  $\alpha_2$ -MFP responses. These data suggest that a balance between the neurotransmitters is required for certain of the metabolic changes after icv LEM without affecting the fever response. However, antipyretics appear to influence only the fever response associated with icv LEM but not the metabolic changes.



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TABLE I. EFFECTS OF ATROPINE,  $\alpha$ -METHYL-P-TYROSINE, p-CHLOROPHENYLALANINE  
ON THE LEM RESPONSE.

LEM	Time (hr)	Zinc ( $\mu$ g/100 ml)	$\alpha_2$ -Macrofetoprotein (Units/ml)
Alone	0	142 $\pm$ 4 <sup>a</sup>	
	5	68 $\pm$ 2 <sup>†</sup>	0.2 $\pm$ 0.2
	24	88 $\pm$ 8 <sup>‡</sup>	22.0 $\pm$ 0.5 <sup>‡</sup>
+ Atropine	0	138 $\pm$ 8	0
	5	78 $\pm$ 11 <sup>†</sup>	0
	24	83 $\pm$ 9 <sup>†</sup>	6.4 $\pm$ 4.0*
+ $\alpha$ -MPT	0	141 $\pm$ 3	0.5 $\pm$ 0.2
	5	59 $\pm$ 11 <sup>†</sup>	0.7 $\pm$ 0.5
	24	71 $\pm$ 9 <sup>†</sup>	5.2 $\pm$ 3.0**
+ p-Chlorophenylalanine	0	152 $\pm$ 4	1.8 $\pm$ 1.0
	5	64 $\pm$ 9 <sup>†</sup>	2.2 $\pm$ 1.0
	24	66 $\pm$ 8 <sup>†</sup>	7.7 $\pm$ 2.0**

<sup>a</sup> Each value is the mean  $\pm$  SE.

\* $\underline{P}$  < 0.05 compared to LEM alone.

\*\* $\underline{P}$  < 0.01 compared to LEM alone.

<sup>†</sup> $\underline{P}$  < 0.01 compared to 0 time.

<sup>‡</sup> $\underline{P}$  < 0.001 compared to 0 time.

TABLE II. EFFECTS OF APAP, ASPIRIN AND INDOMETHACIN ON THE LEM RESPONSE

LEM	Time (hr)	Zinc ( $\mu\text{g}/100\text{ ml}$ )	$\alpha_2$ -Macroglobulin (units/ml)
Alone	0	$150 \pm 10^a$	0
	5	$71 \pm 2^{**}$	0
	24	$91 \pm 8^*$	$21.0 \pm 7^{**}$
+ APAP	0	$147 \pm 8$	0
	5	$60 \pm 8^{**}$	0
	24	$72 \pm 7^{**}$	$17.0 \pm 4^{**}$
+ Aspirin	0	$135 \pm 4$	0
	5	$61 \pm 6^{**}$	0
	24	$78 \pm 3^{**}$	$16.0 \pm 3^{**}$
+ Indomethacin	0	$138 \pm 3$	0
	5	$63 \pm 2^{**}$	0
	24	$74 \pm 3^{**}$	$15.0 \pm 0^{**}$

<sup>a</sup>Each value is the mean  $\pm$  SE.

\* $p < 0.01$  compared to 0 time.

\*\* $p < 0.001$  compared to 0 time.

## LEGEND TO FIGURES

Fig. 1. Effects of pretreatment with  $\alpha_2$ -MPT PCPA and atropine on the LEM (20  $\mu$ l) induced fever. Arrow indicates the administration of LEM. Each point represents the mean  $\pm$  SE of 8-10 rats.

Fig. 2. Effect of antipyretic agents (APAP aspirin, and indomethacin) on the fever response produced by icv injections of LEM. First arrow indicates the administration of LEM, while the second arrow represents the antipyretic injection. LEM alone group received an injection of saline. Each point represents the mean  $\pm$  SE of 8-10 rats. Significance of difference from LEM treatment,  $**P < 0.01$ .

Fig. 3. Effect of pretreatment with antipyretic agents (APAP, aspirin, and indomethacin) on the fever produced by icv injections of LEM. Arrow indicates the administration of LEM. Each point represents the mean  $\pm$  SE of 8-10 rats. Significance of difference from LEM treatment,  $*P < 0.05$ .



Fig. 1

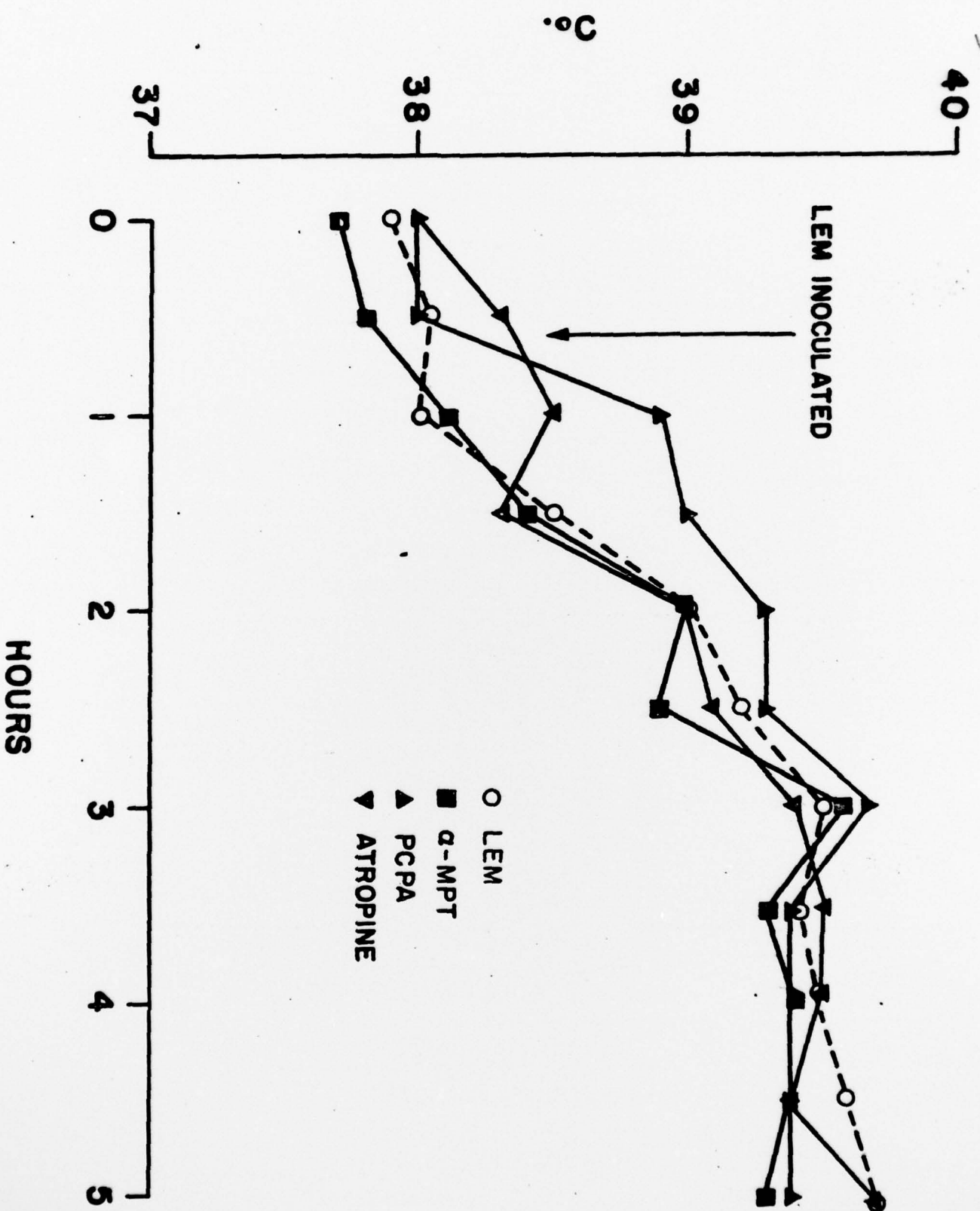


Fig. 2

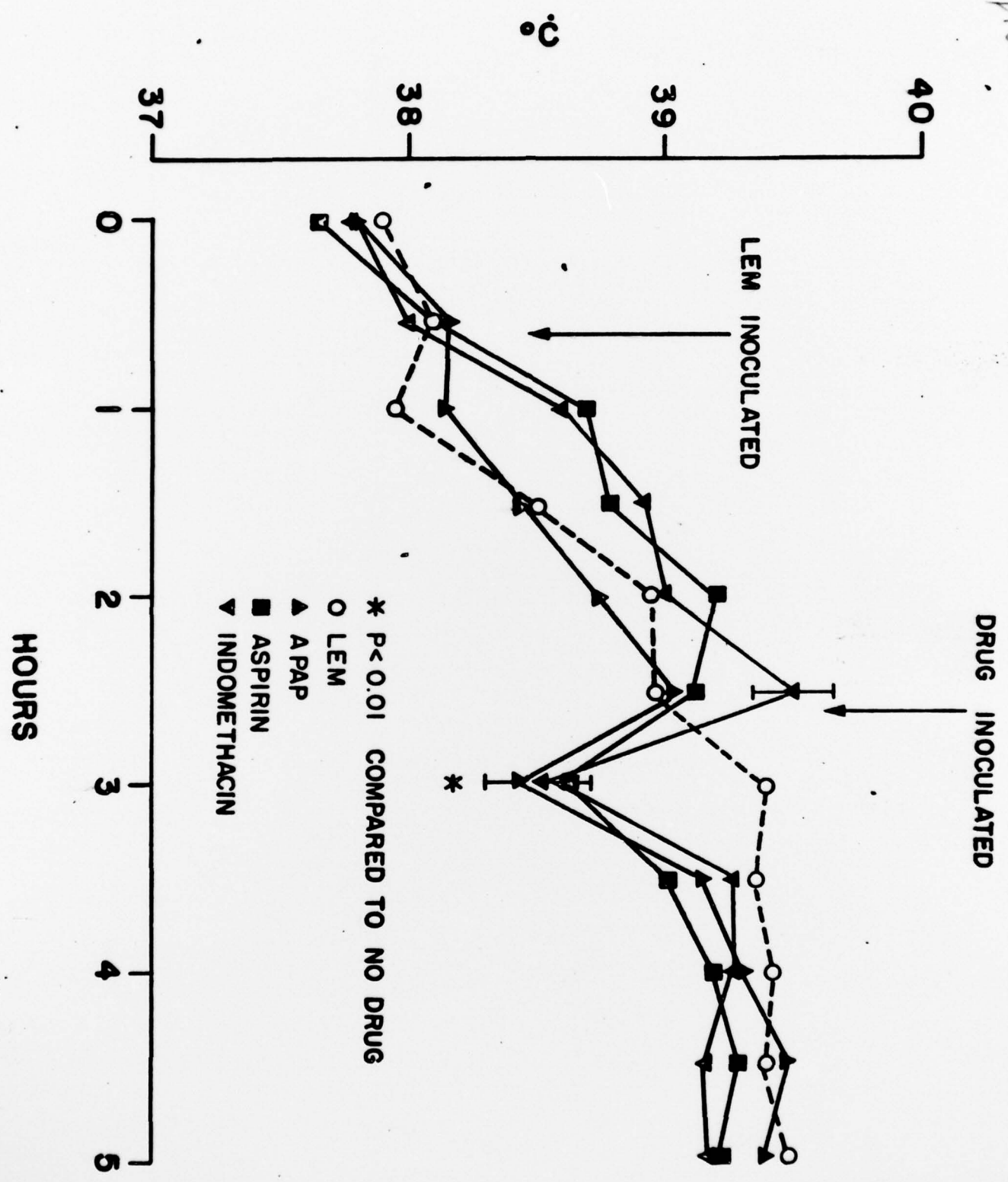


Fig. 3

